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## **Exploring early life events including diet in cats presenting for gastrointestinal signs in later life**

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## Abstract

Our study aimed to determine if certain early life events were more prevalent in cats presenting to veterinary practices specifically for gastrointestinal signs on at least 2 occasions between 6 and 30 months of age. Data from an owner-completed questionnaire for 1,212 cats before 16 weeks of age and subsequent questionnaires for the same cats between 6 and 30 months of age were reviewed.

Of the 1,212 cats included, 30 visited a veterinary practice for gastrointestinal signs on two or more occasions. Of the early life events recorded, cats reported with vomiting, diarrhoea or both and/or those not exclusively fed commercial diet(s) that meet the WSAVA Global Nutrition Committee (GNC) guidelines before 16 weeks of age were more likely to visit veterinary practices specifically for gastrointestinal signs on at least two occasions between 6 and 30 months of age ( $p < 0.001$ , odd's ratio (OR)=2.64, 95% confidence interval (CI)=1.66-4.22 and  $p = 0.030$ , OR=1.51, 95% CI=1.04-2.22, respectively).

Ensuring cats exclusively consume commercial diet(s) that meet the WSAVA GNC guidelines, and further studies identifying specific aetiologies for vomiting and diarrhoea before 16 weeks of age to enable prevention may reduce the number of cats subsequently presenting to primary care veterinary practices for repeated gastrointestinal signs.

## Introduction

The exposome is a term that has been used to describe the sum of all environmental factors a human is exposed to during their lifetime, beginning *in utero* and ending at death (1). The exposome together with genetic susceptibility may impact the intestinal microbiota and mucosal immune system resulting in chronic relapsing gastrointestinal disease (2). There is increasing evidence that early life exposures in humans are important to chronic disease risk later in life (3, 4). In addition, studies have shown that a number of early life factors may influence the development of immune tolerance as well as the composition of the intestinal microbiota (5-7). Therefore, many studies have specifically focused on identifying early life risk factors for the development of chronic relapsing gastrointestinal diseases in humans, such as inflammatory bowel disease (IBD) and functional gastrointestinal disorders (8-11). Identification of such factors, particularly in early life is important to increase our understanding of disease pathogenesis as well as enable disease prevention and treatment.

The effect of the exposome on an animal's chronic disease risk has very rarely been studied: one study showed that dogs that survive canine parvovirus infection had a significantly higher risk of developing chronic gastrointestinal signs later in their lives (12). Currently no studies exist assessing early life events in cats that may serve as risk factors for subsequent chronic

gastrointestinal signs. Therefore, this study aimed to identify if certain events in early life were more prevalent in cats presenting at least twice to primary care veterinary practices specifically for gastrointestinal signs between 6 and 30 months of age compared to those cats that had never visited for gastrointestinal signs. Identification of potential early life risk factors may help in the prevention of repeated gastrointestinal signs in cats.

## Materials and Methods

### Cats

Cats used in this study were those that had previously been prospectively enrolled onto the Bristol Cats Study, a long-term longitudinal study of cat health, welfare and behaviour. Owners in the United Kingdom were recruited onto the study between May 1, 2010 and December 31, 2013. During 2010, enrolment was restricted to owners who lived in Bristol, and then was extended to the whole of the United Kingdom from January 1, 2011 in order to increase numbers.

### Data collection

Owners were asked to complete a questionnaire at enrolment onto the Bristol Cats Study when

their cat was between 8 and 16 weeks of age (Q1). The owners of the same cats completed subsequent questionnaires when their cats reached the ages of 6 months (Q2), 12 months (Q3), 18 months (Q4), 30 months (Q5), 48 months (Q6) and then at annual time intervals. Data for our study were taken from the first 5 questionnaires (Q1-5). Questionnaires were available to participating owners either in an online or in paper format. Most questions were presented in a multiple-choice format and questionnaires took approximately 10 to 15 minutes to complete. Further details regarding cohort recruitment and questionnaire design and distribution can be found in Murray et al., 2017 (13) and in the supplemental data for that manuscript.

#### Cases and controls

Cases were defined as those cats that were reported by their owners to have visited a veterinary practice, specifically for gastrointestinal signs (vomiting, diarrhoea or both) at one or all of the 4 time-points (Q2, Q3, Q4, Q5). The questionnaires did not specifically address the diagnostic investigation, definitive or tentative diagnosis or treatment at these visits. Controls were those cats that were reported by their owners to have never visited a veterinary practice specifically for gastrointestinal signs (vomiting, diarrhoea or both) at all of the 4 time-points (Q2, Q3, Q4, Q5).

#### Potential early life risk events

Questionnaire 1, which had been completed when the cats were between 8 to 16 weeks of age was reviewed for all cats in the case and control groups and only information on the following 4 early life events were recorded for each: 1) name of commercial diets fed and whether each complied with the World Small Animal Veterinary Association (WSAVA) Global Nutrition Committee (GNC) guidelines, 2) proportion of diet fed that consisted of raw fresh food, cooked fresh food and cow's milk or cream (owners could select one of 5 options: all of the diet,  $\geq 50\%$  of diet,  $< 50\%$  of diet, occasionally or never), 3) owner reported vomiting, diarrhoea or both and 4) presence of owner reported helminths in the faeces.

To determine whether the commercial diet each cat was consuming complied with the WSAVA GNC guidelines, each manufacturer was contacted and asked to reply to the 8 questions outlined in section A of the web link<sup>1</sup>. Diets were considered to meet the WSAVA GNC guidelines if the manufacturer could satisfactorily address all of the questions.

### Ethical considerations

The University of Bristol granted ethical approval for the study (VIN/17/049).

### Data analysis and statistics

Analyses were performed using a computer software package (IBM SPSS Statistics Version

23). Univariable and multivariable ordinal regression models were constructed to assess associations between the following 4 early life events, identified before 16 weeks of age: 1) whether cats exclusively consumed commercial diet(s) that complied with the WSAVA GNC guidelines, 2) proportion of diet fed that was raw fresh food, cooked fresh food and cow's milk or cream, 3) owner reported vomiting, diarrhoea or both, 4) presence of owner reported helminths in the faeces and the frequency of owner reported visits to veterinary practice specifically for gastrointestinal signs between 6 and 30 months of age. Feeding exclusively commercial diet(s) that complied with the WSAVA GNC guidelines, owner reported vomiting, diarrhoea or both and the presence of owner reported helminths in the faeces were entered into the general linear models as yes/no. The proportion of diet fed that consisted of raw fresh food, cooked fresh food and cow's milk or cream were entered into the general linear model as categorical data with 1 representing all of the diet, 2 as 50% or more of the diet, 3 as less than 50% of the diet, 4 as occasionally and 5 as never. Variables associated with the frequency of owner reported visits to veterinary practices specifically for gastrointestinal signs between 6 and 30 months of age with p-value <0.2 in the univariable analysis were entered into the multivariable analyses. In the multivariable regression models, analyses were performed in a backward stepwise manner. All variables were initially included, and the variable with the



highest p-value was removed until all remaining variables had a p-value  $<0.05$ .

Multicollinearity was assessed by evaluating the correlation matrix. Odd's ratios (OR) with 95% confidence intervals (CI) were also calculated.

## Results

### Cats

One thousand two hundred and twelve cats that were initially enrolled onto the study between 8 and 16 weeks of age had all subsequent questionnaires (Q2-Q5), from 6 to 30 months of age available for review.

One thousand and fourteen cats were reported to have never visited a veterinary practice specifically for gastrointestinal signs between 6 and 30 months of age. One hundred and sixty eight cats were reported by their owners to have visited a veterinary practice on one occasion specifically for gastrointestinal signs between 6 and 30 months of age. A separate 26 cats were seen twice, of which 12 cats were reported to have visited at both the 18 month and 30 month questionnaire, 7 cats at both the 12 month and 18 month questionnaire, 5 cats at both the 12 month and 30 month questionnaire, of which one was reported to be diagnosed with inflammatory bowel disease on intestinal histopathology, one cat at both the 6 month and 12

month questionnaire and one cat at both the 6 month and 18 month questionnaire. A separate 4 cats visited 3 times, with all 4 reported to have visited at the 12 month, 18 month and 30 month questionnaire (figure 1).

Early life events associated with the frequency of visits to a veterinary practice specifically for gastrointestinal signs between 6 and 30 months of age.

In the univariable analyses, the proportion of diet fed that consisted of raw fresh food, cooked fresh food, cow's milk or cream and the presence of owner reported helminths in the faeces before 16 weeks of age did not significantly affect the frequency of cat visits to veterinary practices specifically for gastrointestinal signs between 6 and 30 months of age ( $p > 0.05$ , table 1). Cats with reported vomiting, diarrhoea or both or those not exclusively fed commercial diet(s) that meet the WSAVA Global Nutrition Committee (GNC) guidelines before 16 weeks of age were significantly associated with the frequency of visits to a veterinary practice specifically for gastrointestinal signs between 6 and 30 months of age in the univariable analyses ( $p < 0.001$ , OR=2.60, 95% CI=1.64-4.26 and  $p = 0.038$ , OR=1.49, 95% CI=1.23-2.17, respectively, table 1).

The proportion of raw fresh food fed, reported vomiting, diarrhoea or both and whether cats were exclusively consuming commercial diet(s) that meet the WSAVA GNC guidelines before

16 weeks of age were analysed further in the final multivariable model, as these variables had a p-value of less than 0.2 in the univariable analyses (table 1). The multivariable model showed that vomiting, diarrhoea or both and/or those not exclusively fed commercial diet(s) that meet the WSAVA Global Nutrition Committee (GNC) guidelines before 16 weeks of age were significantly associated with the frequency of visits to a veterinary practice specifically for gastrointestinal signs between 6 and 30 months of age ( $p < 0.001$ , OR=2.64, 95% CI=1.66-4.22 and  $p = 0.030$ , OR=1.51, 95% CI=1.04-2.22, respectively, table 2).

## Discussion

Environmental factors are important triggers for many chronic gastrointestinal diseases in humans, including IBD (14). Our study for the first time demonstrated early life events that may act as significant risk factors for cats that subsequently visit veterinary practices specifically for gastrointestinal signs on two or more occasions between 6 and 30 months of age. Our study identified owner-reported vomiting, diarrhoea or both and/or those not exclusively fed commercial diet(s) that meet the WSAVA Global Nutrition Committee (GNC) guidelines before 16 weeks of age as being potentially significant risk factors for cats presenting to veterinary practices specifically for gastrointestinal signs on two or more occasions between 6 and 30 months of age.

Infectious gastroenteritis has been shown to be a significant risk factor for the subsequent development of IBD and functional gastrointestinal disorders in humans (15, 16). Similarly, dogs that survive canine parvovirus infection have a significantly higher risk of developing chronic gastrointestinal signs later in their lives (12). Unfortunately, the aetiology of vomiting and diarrhoea before 16 weeks of age in the cats in our study was unknown. However, in humans the presence of an acute inflammatory response in the gastrointestinal tract rather than a specific aetiological pathogen may be responsible for the subsequent development of IBD (15). Therefore, the presence of gastrointestinal inflammation causing episodes of vomiting and diarrhoea regardless of disease aetiology in our cats before 16 weeks of age may trigger an initial over compensated response, which may then lead to over activation of the intestinal mucosal immune system leading to chronic inflammation. Alternatively, episodes of gastrointestinal inflammation before 16 weeks of age may cause lasting changes in the microbiota or gut epithelial barrier, which may subsequently increase susceptibility to gastrointestinal inflammation, resulting in these cats being frequently presented to veterinary practices for their signs. Therefore, determining the aetiology of vomiting and diarrhoea in cats before 16 weeks of age may help to identify areas for prevention, which may then reduce the

number of cats presenting to veterinary practices specifically for repeated gastrointestinal signs.

In this study, cats that were not exclusively fed commercial diet(s) that meet the WSAVA Global Nutrition Committee (GNC) guidelines before 16 weeks of age were more likely to subsequently present to veterinary practices specifically for gastrointestinal signs on two or more occasions. The WSAVA GNC guidelines outlines 8 questions for manufacturers of the diet to help ensure that a reputable and knowledgeable company that uses strict quality control measures formulates the food. According to the WSAVA GNC guidelines, if the manufacture is not able to answer the 8 questions satisfactorily, then owners should be cautious with feeding that brand. The results of our study further justify the need to ensure that cats are exclusively receiving commercial diet(s) that meets the WSAVA GNC guidelines before 16 weeks of age. Feeding a diet that does not comply with the WSAVA GNC guidelines may raise concerns regarding the source of ingredients, nutritional composition and quality control of the diet, factors which may impact on the gastrointestinal mucosal immune system, microbiota and intestinal permeability. However, further studies would be needed to determine if any attributes of these diets are specifically implicated in disease pathogenesis.

Pre-illness dietary risk factors such as increased fat has repeatedly been shown to predispose to IBD in humans (19, 20). Unfortunately, due to the time frame of this study and the lack of detailed information regarding flavours of commercial diets fed, it was not possible to determine specific macronutrient profiles of diets fed before 16 weeks of age that may be associated with the frequency of cats presenting to veterinary practices specifically for gastrointestinal signs. In addition, as this study specifically focused on early life events, dietary changes and commercial diets that were fed prior to cats presenting to veterinary practices for gastrointestinal signs during the 30 month study period was not specifically assessed. Another limitation of this study was that the underlying definitive diagnosis for the gastrointestinal signs for which cats were presented to veterinary practices was unknown as medical records were unavailable for review for all cats. However, one owner commented on the questionnaire that their cat was diagnosed with IBD on intestinal biopsy at one of the visits. However, all cats in this study are continuing to be monitored with annual questionnaires and therefore in the future this may reveal definitive diagnoses for their gastrointestinal signs. In addition, as our study only included questionnaires until 30 months of age, cats that present later in life to veterinary practices specifically for repeated gastrointestinal signs will be missed. Therefore, a follow-up study to assess these cats when they reach 10 to 12 years of age or have a definitive diagnosis

for their gastrointestinal signs may give us more definitive information with regards to which early life events are significantly associated with chronic gastrointestinal disease. A follow-up study may also allow the inclusion of more cats that had presented to veterinary practices specifically for gastrointestinal signs on two or more occasions, which may allow for stronger conclusions to be made regarding early life events in these cats. Also, as the medical records were unavailable for the cats, the authors were unable to rule out any association of the significant early life events identified in this study with additional non-gastrointestinal diseases or conditions in these cats. Therefore, further studies are likely warranted to determine if owner reported vomiting, diarrhoea or both and/or not exclusively feeding commercial diet(s) that meet the WSAVA GNC guidelines before 16 weeks of age are associated with additional non-gastrointestinal diseases or conditions in cats. A final limitation of this study was additional early life risk factors that have been identified in humans with chronic gastrointestinal diseases, such as antibiotic use, early weaning and caesarean delivery were not investigated in our study. Studies have implicated antibiotic use in childhood with subsequent increased risk of developing IBD (10, 18). Unfortunately, our study was unable to determine if antibiotic use in early life increased the risk of cats presenting to veterinary practices, as this information was unavailable from questionnaire 1. Therefore, future studies should specifically aim to

determine if antibiotic use in early life affects the frequency of cats presenting to veterinary practices specifically for gastrointestinal signs. Determining if antibiotic use early in life predisposes to the development of repeated gastrointestinal signs in cats is important as this may ensure increased discretion of when to prescribe antibiotic therapy.

In conclusion, our study for the first time showed that cats that had reported vomiting, diarrhoea or both and/or those not exclusively fed commercial diet(s) that meet the WSAVA Global Nutrition Committee (GNC) guidelines before 16 weeks of age were more likely to visit veterinary practices specifically for gastrointestinal signs on two or more occasions between 6 and 30 months of age. Therefore, ensuring that cats exclusively consume a commercial diet that meets the WSAVA GNC guidelines, particularly before 16 weeks of age and further studies identifying specific aetiologies for vomiting and diarrhoea before 16 weeks of age to allow for prevention may reduce the number of cats presenting to primary care veterinary practices specifically for repeated gastrointestinal signs.

#### Footnotes

<sup>1</sup><http://www.wsava.org/sites/default/files/Recommendations%20on%20Selecting%20Pet%20Foods.pdf>.



### Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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**Table 1 - Univariable ordinal regression analyses for early life events in cats subsequently presenting to veterinary practices for gastrointestinal (GI) signs between 6 and 30 months**

**of age.** Table 1 illustrates the number and percentage of cats presenting with early life events

before 16 weeks of age and the frequency of subsequent visits to veterinary practices specifically for GI signs between 6 and 30 months of age. P-values are displayed for the different early life events using univariable ordinal regression analyses. For raw fresh food, cooked fresh food and cow's milk/cream: 1- all of the diet, 2 -  $\geq 50\%$  of the diet, 3 -  $< 50\%$  of the diet, 4 – occasionally in the diet and 5 – never in the diet.

Early life event before 16 weeks of age		Never visited a vet practice for GI signs between 6 and 30 months of age. Number (%)	Visited vet practice for GI signs on 1 occasion between 6-30 months of age. Number (%)	Visited vet practice for GI signs on 2 occasions between 6-30 months of age. Number (%)	Visited vet practice for GI signs on 3 occasions between 6-30 months of age. Number (%)	P-value
Vomiting, diarrhoea or both	Yes	64 (6.3)	23 (13.7)	5 (19.2)	1 (25.0)	<0.001
	No	950 (93.7)	145 (86.3)	21 (80.8)	3 (75.0)	
Helminths in faeces	Yes	43 (4.3)	12 (7.1)	0 (0.0)	1 (25.0)	0.203
	No	946 (95.7)	156 (92.9)	26 (100)	3 (75.0)	
Raw fresh food	1	6 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0.069
	2	31 (3.5)	2 (1.5)	0 (0.0)	0 (0.0)	
	3	81 (9.0)	11 (8.0)	3 (13.0)	1 (25.0)	
	4	354 (39.5)	50 (36.5)	8 (34.8)	1 (25.0)	
	5	424 (47.3)	74 (54.0)	12 (52.2)	2 (50.0)	
Cooked fresh food	1	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.970
	2	7 (0.8)	2 (1.5)	0 (0.0)	0 (0.0)	
	3	22 (2.5)	5 (3.6)	0 (0.0)	2 (50.0)	
	4	145 (16.5)	18 (13.1)	2 (9.1)	0 (0.0)	

	<b>5</b>	702 (80.1)	112 (81.8)	20 (90.9)	2 (50.0)	
<b>Cow's milk/cream</b>	<b>1</b>	4 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0.404
	<b>2</b>	16 (1.7)	1 (0.7)	1 (4.2)	0 (0.0)	
	<b>3</b>	96 (10.5)	16 (11.0)	2 (8.3)	0 (0.0)	
	<b>4</b>	379 (41.4)	57 (39.3)	11 (45.8)	1 (25.0)	
	<b>5</b>	420 (46.0)	71 (49.0)	10 (41.7)	3 (75.0)	
<b>Exclusively feeding commercial diet(s) that meet(s) the WSAVA Global Nutrition Committee Guidelines</b>	<b>Yes</b>	757 (82.1)	118 (75.6)	18 (75.0)	3 (75.0)	0.038
	<b>No</b>	165 (17.9)	38 (24.4)	6 (25.0)	1 (25.0)	

**Table 2 - Multivariable ordinal regression analyses for early life events in cats**

**subsequently presenting to veterinary practices for gastrointestinal (GI) signs between 6**

**and 30 months of age.** Table 2 illustrates the P-values with odd's ratio (OR) and 95%

confidence intervals (CI) for those early life events before 16 weeks of age that were

significantly associated with the frequency of visits to a veterinary practice specifically for GI

signs between 6 and 30 months of age following multivariable ordinal regression analyses.

Early life event before 16 weeks of age		Never visited a vet practice for GI signs between 6 and 30 months of age. Number (%)	Visited vet practice for GI signs on 1 occasion between 6-30 months of age. Number (%)	Visited vet practice for GI signs on 2 occasions between 6-30 months of age. Number (%)	Visited vet practice for GI signs on 3 occasions between 6-30 months of age. Number (%)	P-value (OR, 95% CI)
Vomiting, diarrhoea or both	Yes	64 (6.3)	23 (13.7)	5 (19.2)	1 (25.0)	<0.001 (2.64, 1.66-4.22)
	No	950 (93.7)	145 (86.3)	21 (80.8)	3 (75.0)	
Exclusively feeding commercial diet(s) that meet(s) the WSAVA Global Nutrition Committee Guidelines	Yes	757 (82.1)	118 (75.6)	18 (75.0)	3 (75.0)	0.030 (1.51, 1.04-2.22)
	No	165 (17.9)	38 (24.4)	6 (25.0)	1 (25.0)	



**Figure 1 - A bar chart illustrating the number of cats that visited a veterinary practice specifically for gastrointestinal signs according to the owner between 6 and 30 months of age.**